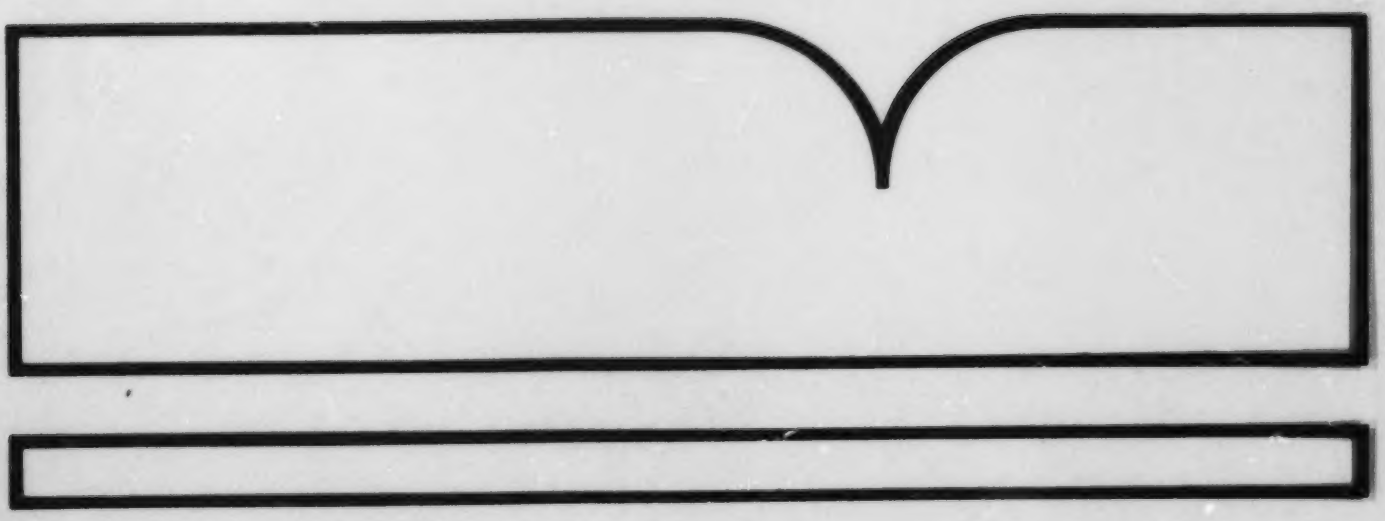
PB84-168657

Metabolism and Genotoxicity of 1-Nitropyrene

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1984



U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-168657

EPA-600/D-84-086
1984

METABOLISM AND GENOTOXICITY OF 1-NITROPYRENE

by

L. M. Ball and J. Lewtas
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

EPA Project Officer
Joellen Lewtas

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/D-84-086	2.
3. RECIPIENT'S ACCESSION NO. PB84-168657	
4. TITLE AND SUBTITLE Metabolism and genotoxicity of 1-nitropyrene	5. REPORT DATE 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) L. M. Ball and J. Lewtas	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Health Effects Research Laboratory Genetic Toxicology Division, Genetic Bioassay Branch (MD 68), Research Triangle Park, NC 27711	10. PROGRAM ELEMENT NO. A9RA1B
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Health Effects Research Laboratory, Genetic Toxicology Division, Genetic Bioassay Branch (MD 68) Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

1-Nitropyrene (NP), a nitrated polycyclic aromatic hydrocarbon and a potent bacterial mutagen, has been identified in combustion emissions and may contribute to the burden of genotoxicity associated with air pollution. NP undergoes rapid metabolism by rat hepatic subcellular fractions (S-9, cytosol and microsomes) and isolated cells to both oxidised and reduced products. Metabolism is accompanied by extensive binding to endogenous protein and DNA in isolated hepatocytes, and to calf thymus DNA added to the subcellular fractions. Metabolites of NP have been isolated, identified and characterised by HPLC, HRGC/MS and chemical synthesis. In the intact rat NP is excreted in the urine as phenols of 1-nitropyrene (1-NP-6/8- and -3-OH) and of N-acetyl-1-aminopyrene (NAAP-6- and -8-OH), all extensively conjugated with glucuronic acid. Some of these metabolites are mutagens five-fold more potent in the Ames Salmonella assay than the parent compound. Studies in germ-free rats demonstrated that metabolism by the gut flora and enterohepatic recirculation play a vital role in the production of the highly mutagenic NAAP-6/8-OH. Metabolic species capable of causing genotoxic damage can thus be produced by mammalian mechanisms both alone and in conjunction with bacterial metabolism.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 16
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

METABOLISM AND GENOTOXICITY OF 1-NITROPYRENE

L.M. BALL* and J. LEWTAS
Genetic Bioassay Branch, Health Effects Research Laboratory,
U.S. Environmental Protection Agency, Research Triangle Park,
NC 27711, U.S.A.

INTRODUCTION

Nitroarenes such as 1-nitropyrene (NP; Figure 1), once found in xerographic toners (1,2), have now been identified as highly mutagenic components of extracts of particulate matter

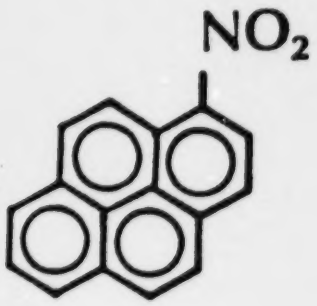


FIGURE 1: Structure of 1-nitropyrene.

derived from diesel emissions (3,4) and indirect evidence suggests that they may be widespread as ambient air pollutants (5). Activation of these compounds in bacterial assays appears to proceed largely through nitro reduction via an arylnitrenium or hydroxylamine intermediate (6,7). Evidence is rapidly accumulating to suggest that in mammalian systems other mechanisms such as metabolism by ring oxidation may play a role in the genotoxicity of nitroarenes (8, 9, 10), which may be of greater importance in attempting to evaluate the contribution of these compounds to the mutagenic and carcinogenic burden associated with airborne pollution.

We describe here a series of studies carried out to evaluate the metabolic fate of 1-nitropyrene in vivo and to assess the relative contribution of mammalian and bacterial enzymes in the generation of reactive metabolites from 1-NP.

*National Research Council Resident Research Associate

MATERIALS AND METHODS

Chemicals

1-Nitro[4,5,9,10-^{14}C]pyrene (^{14}C-NP; 10 mCi/mmole, radiochemical and chemical purity 99.9%), unlabelled NP at the same degree of purity, and nitropyrene phenols were prepared by the Midwest Research Institute, Kansas City, MO. The synthesis, isolation and characterization of the reference compounds have been described in detail elsewhere (11). Other chemicals, reagents and solvents were obtained at the highest degree of purity available from commercial sources, principally Sigma Chemical Co, St. Louis, MO, and Burdick & Jackson, Muskegon, MI.

Animals

Tissue fractions and cells were obtained from male Sprague Dawley rats (Charles River CD-1, Wilmington, MO). Rats were injected i.p. with Aroclor 1254 (500mg/kg) 5 days before sacrifice. The animals used for in vivo studies were Charles River Sprague Dawley male rats, body wt 200-220g, and also male and female rats of the AGUS strain, either maintained under conventional laboratory conditions or raised germ-free in stainless steel isolators by the method of Gustafsson et al (12). After injection with ^{14}C-NP, the rats were kept in stainless steel metabolism cages which allowed separate collection of urine and faeces.

Analytical Techniques

High Pressure Liquid Chromatography (HPLC). HPLC analysis was performed on a Varian Model 5060 Liquid Chromatograph (Varian Associates Inc, Palo Alto, CA) or an LDC system composed of a Rheodyne 7125 injector, Constametric I and III pumps, a Gradient Master controller and a Mixer (Milton Roy Co., Stone, Staffs, England) Analytical separations of NP and its metabolites were achieved on a Zorbax ODS (Dupont Instrument Co, Wilmington, DE) or a μBondapak C-18 (Waters Instrument Co, Boston, MA) 4.6x250mm reverse phase column eluted with a gradient of 60 to 100 % MeOH in water. The eluate was monitored at 280nm (Varian UV-Vis 50 or LDC Spectromonitor III) and collected in scintillation vials for ^{14}C determination by liquid scintillation counting. Figure 2 shows an elution profile for synthetic standards and metabolites of NP.

High Resolution Gas Chromatography(HRGC)/Mass Spectrometry(MS). Samples were methylated with Diazald (Aldrich

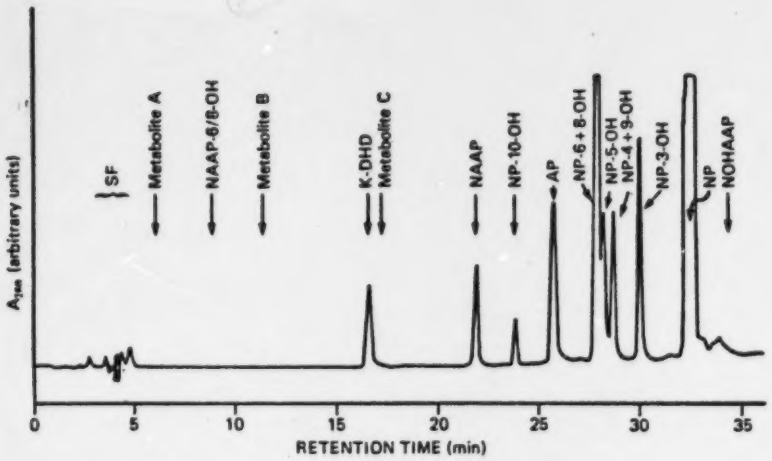


FIGURE 2: HPLC trace of NP and its metabolites. NP and the following synthetic compounds were chromatographed on a reverse phase column (Zorbax-ODS) eluted with a gradient of MeOH in H_2O: NAAP-6/8-OH, 6- or 8-hydroxy-N-acetyl-1-aminopyrene; K-DHD, K-region dihydrodiols of NP; NAAP, N-acetyl-1aminopyrene; NP-10-OH, 10-hydroxy-1-nitropyrene; similarly, five other nitropyrene phenols; AP, 1-aminopyrene; NP, 1-nitropyrene; NOHAAP, N-hydroxy-N-acetyl-1-aminopyrene. The elution profile of the synthetic standards (monitored at 280nm) is shown; the elution points of unidentified *in vivo* metabolites (Metabolites A, B and C) are indicated by arrows. SF, solvent front.

Chemical Co, Milwaukee, WI) then injected at 40°C onto a fused silica capillary column interfaced directly to the ion source of a Finnigan 4000 (Finnigan Corp., Sunnyvale, CA) mass spectrometer operated in the positive chemical ionization mode with CH_4 as the reagent gas. Total ion current and spectra were recorded as the oven temperature was increased from 40°C to 300°C at 10°/min.

Spectrometry. High resolution mass spectra (HRMS) were recorded by direct probe insertion on a VG 7070 mass spectrometer at 70eV in the electron impact mode. NMR data were obtained at 250 or 500 MHz in acetone-d_6.

Mutagenicity Assays

The Salmonella typhimurium plate incorporation assay with and without exogenous metabolic activation, was carried out as described by Ames (13), with modifications (14). Overnight (stationary phase) cultures were used throughout. S. typhimurium strain TA98 was obtained from Dr. B. Ames, University of California, Berkeley, CA. TA98 detects frameshift mutagens, and of the five basic Ames tester strains is the one most responsive to nitroarenes. 2-Nitrofluorene (without activation, 0.5 µg/plate), and 2-anthramine (0.5 µg/plate, with activation) were used as positive controls; historical values in this laboratory are 180 to 400 rev/plate and 700 to 1000 rev/plate respectively for these positive controls, and experiments falling outside these ranges were rejected.

S9 to provide exogenous metabolic activation was obtained from the livers of Aroclor-treated rats; the amount added per plate to provide exogenous metabolic activation was optimized for response to benzo[a]pyrene, 2-anthramine and 7,12-dimethylbenz[a]anthracene. The NADPH-generating system consisted of glucose-6-phosphate, 5mM; NADP, 4mM, sterilized by passing through a 0.45 µm filter; $MgCl_2.6H_2O$, 8mM; KCL, 26.25 mM; 0.2 M phosphate buffer pH 7.4; and sterile distilled H_2O so that the final solution (after addition of S9 fraction) was 0.1 M with respect to phosphate.

Chemicals were dissolved in DMSO for assay. Urine samples were sterilized by passing through a 0.45 µm filter. Plates were counted on an Artek Model 880 Colony Counter (Farmingdale, NY). Mutagenic response was calculated from simple linear regression over a range of at least 4 doses where the sample showed little or no toxicity to the micro-organisms, judged by examination of the background lawn. For potency comparison between compounds, values were expressed as rev/nmol, since the doses used ranged approximately from 0.4 to 40 nmoles.

Incubation Conditions

Freshly-isolated hepatocytes from Aroclor-induced rats (15) were incubated with ^{14}C-NP (50 µM) for 15 min to 2 hr at 37°C in Dulbecco's phosphate-buffered saline medium. Cells were harvested by centrifugation and lysed with sodium dodecyl sulphate. Tissue fractions (S9, cytosol and microsomes) from the livers of untreated and Aroclor-treated rats were suspended in 0.1 M HEPES buffer (1-2 mg protein/ml) containing 2mg/ml of calf thymus DNA (Sigma Chemical Co, St. Louis, MO), and incubated with 100 µM ^{14}C-NP. Reactions were initiated by addition

of 1mM NADPH, and terminated with addition of chloroform-iso-amyl-alcohol-phenol (CIP) saturated with H_2O.

Macromolecular Binding

DNA and protein were isolated from incubated tissue fractions or lysed cell preparations by the CIP extraction technique, and ^{14}C bound to the macromolecules was quantitated by liquid scintillation counting of the solubilised protein or of the DNA precipitated onto glass fiber microfilters, as described fully elsewhere (16).

RESULTS

1-Nitropyrene administered to rats by ip injection (10 mg/kg) was rapidly and extensively metabolised then excreted via both urine and faeces (Figure 3). Potent S9-dependent

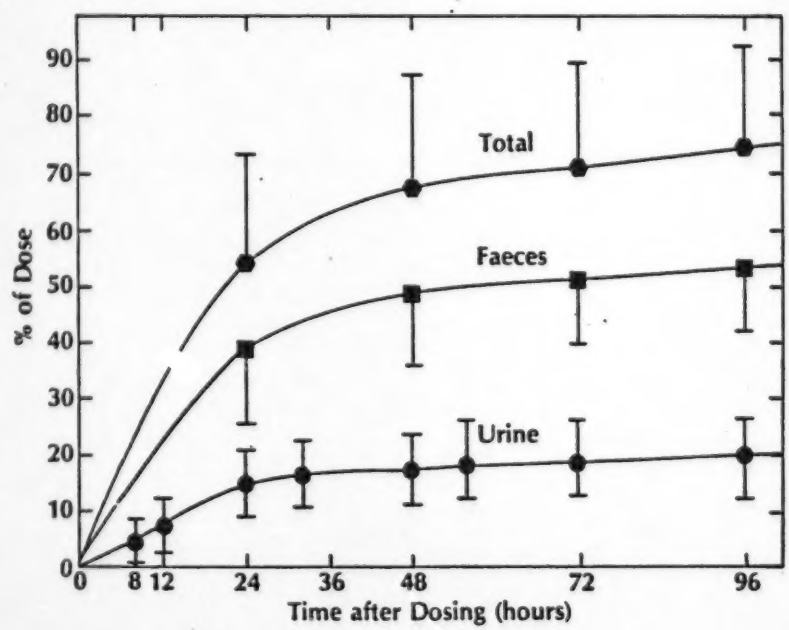


FIGURE 3: Excretion of ^{14}C by rats after dosing with ^{14}C-NP. 4 Rats were injected i.p. with ^{14}C-NP (10mg/kg; 6μCi each). Urine and faeces were collected over the time indicated, and excretion of ^{14}C determined by liquid scintillation counting.

mutagenic activity was detected in the urine by means of the Ames assay. HPLC analysis indicated that originally all the ^{14}C excreted into the urine was in the form of highly polar, water-soluble metabolites. Incubation of urine samples with

TABLE 1

DISTRIBUTION OF 1-NP METABOLITES IN RAT URINE AT SPECIFIED TIMES AFTER DOSING

Metabolite Fraction[a]	Percent of urinary ^{14}C at 0-4 h	12-24 h
Solvent Front (unknown)	51.9	58.0
Metabolite A	<0.5	0.5
NAAP-6/8-OH	1.5	25.0
Metabolite B	<0.5	10.0
Metabolite C	<0.5	<0.5
Dihydrodiol	21.5	1.5
NAAP	5.0	<0.5
Metabolite D	0.7	0.5
Aminopyrene	<0.5	<0.5
NP-6/8-OH	11.0	2.0
NP-3-OH	6.2	<0.5
1-Nitropyrene	1.7	<0.5
Total ^{14}C content (nmol)	134	700
Specific Mutagenicity +S9	24±4	142±39

[a]Urines from rats injected with ^{14}C-NP were analysed for NP metabolites as described.

β-glucuronidase and sulphatase allowed the separation (Figure 2) and quantitation (Table 1) of urinary metabolites of ^{14}C-NP as their deconjugated principals. At all time-points the predominant metabolite fraction consisted of material eluting at the solvent front, which was refractory to enzymic hydrolysis and was not further characterized at this point since its specific mutagenicity was low (10-20 rev/nmole). Ring-hydroxylated derivatives of 1-nitropyrene (6- or 8-hydroxy-1-nitropyrene, NP-6/8-OH, and 3-hydroxy-1-nitropyrene, NP-3-OH) and of N-acetyl-1-aminopyrene (6- or 8-hydroxy-N-acetyl-1-aminopyrene, NAAP-6/8-OH) were identified in urine by comparison of their chromatographic characteristics and mass spectra with those of synthetic reference compounds (Table 2). NAAP-6/8-OH represented only a few percent of the urinary ^{14}C in the first hours

TABLE 2

CHROMATOGRAPHIC AND MASS SPECTRAL DATA ON URINARY METABOLITES OF 1-NITROPYRENE

Compound	HPLC R_T(Min)	HRGC R_T(Min)	m/e of major ions (HRGC/MS-PCI)
NAAP-6/8-OH[a]	8.5	31'42"	290 (100%; $[M+H]^+$)
Metabolite	8.5	31'43"	290 (100%; $[M+H]^+$)
NP-6-OH + NP-8-OH	26	29'29", 29'35"	278, 248, 247
Metabolite	26	29'35"	278, 248, 247
NP-3-OH	29	28'40"	278, 248, 247
Metabolite	29	28'33"	278, 248, 247

[a]Synthetic reference compounds and metabolites isolated from the urine of rats injected with ^{14}C-NP were analyzed by HPLC and HRGC as described under Materials and Methods. Samples were methylated for HRGC and HRGC/MS-PCI.

after dosing, but rose to 25%, the largest identified metabolite fraction, at 12-24h (Table 1). By HPLC fractionation of the urines, NAAP-6/8-OH was shown to be the principal source of urinary mutagenicity, though the phenols of NP itself were also

active in the Ames test, with or without S9 (Table 3).

TABLE 3

MUTAGENICITY OF 1-NITROPYRENE DERIVATIVES

Compound	Mutagenicity towards *Salmonella* TA98	
	-S9	+S9
3-Hydroxy-1-Nitropyrene	103[a]	36
6-Hydroxy-1-Nitropyrene	22	87
8-Hydroxy-1-Nitropyrene	31	19
4+5+9+10-Hydroxy-1-Nitropyrene mixture	1062	198
6- or 8-Hydroxy-NAAP[b]	---	≈600

[a]Mutagenicity, determined in the Ames *Salmonella typhimurium* plate incorporation assay, as described (13,14), is expressed as rev/nmole, calculated from linear regression over a dose range of 0.05 to 5µg compound/plate.

[b]Metabolite isolated from the urine of rats dosed with ^{14}C-NP.

The role of the gut flora in the generation of mutagenic metabolites was investigated by comparing the metabolism of ^{14}C-NP in germ-free rats of the AGUS strain with that in rats of the same strain with conventional gut flora (17). Urines collected from 8 to 24 h after dosing (the time interval during which maximum excretion of mutagens had been observed previously) were analysed for mutagenicity and by HPLC. The specific mutagenicity (calculated from total ^{14}C content) of the urinary metabolites from germ-free animals was much lower than that from the rats with conventional flora, and as potent without as with the addition of S9 (Figure 4). The NAAP-6/8-OH content of these urines was also much reduced (2% of the ^{14}C in germ-free urine compared to 23% of ^{14}C in conventional urine).

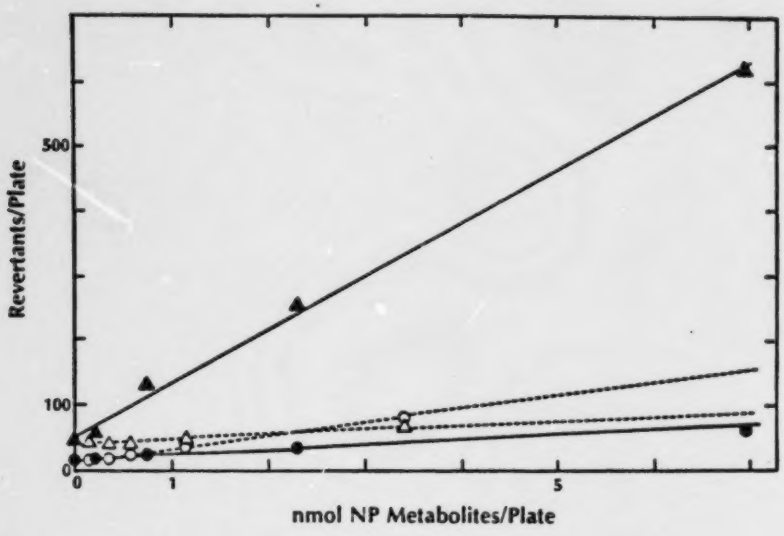


FIGURE 4: Mutagenicity of the urine of germ-free compared to conventional rats. Germ-free and conventional rats of the AGUS strain were injected i.p. with ^{14}C-NP (1.5mg each). Urine and faeces were collected, and the 8-24h urines were assayed for mutagenicity in the Ames plate incorporation system (13,14). —▲—▲— Conventional rat urine with S9; —•—•— conventional rat urine without S9; --Δ-- germ-free rat urine with S9; --o-- germ-free rat urine without S9.

Incubation with freshly isolated hepatocytes and liver subcellular fractions showed that liver enzymes were capable of activating 1-NP to intermediates that bound to DNA (Table 4). In both microsomes and S9, aerobic incubations resulted in more extensive binding than did incubations conducted under N_2. NADPH was required for DNA binding to occur in microsomes or S9, while cytosolically-catalysed binding was much lower, and essentially independant of NADPH (results not shown), as it was of air. Aroclor treatment results in substantial increase in the binding levels seen in both microsomes and S9. These results point to an involvement of mixed-function oxidase activity in the generation of active intermediates from ^{14}C-NP capable of interacting with DNA, but the lack of a clear-cut difference in specific activity between S9 and microsomes also suggests that some degree of interaction between cytosolic and microsomal pathways may be required for activation.

TABLE 4

BINDING OF ^{14}C-NP TO ENDOGENOUS OR EXOGENOUS DNA, CATALYSED BY RAT HEPATIC ENZYMES

Enzyme Source[a]	Treatment: None		Aroclor	
	Incubation under: Air	N_2	Air	N_2
Cytosol	0.5± 0.1[b]	0.6±0.1	3.2±3.0	1.8±1.6
Microsomes	43±12	13±7	125±54	54±29
S9	21±3	8±2	246±55	33±16
Hepatocytes	----------		5.6±0.8[c]	----

[a]Tissue fractions were obtained from untreated or Aroclor-treated rats as described in Materials and Methods.

[b]DNA binding was determined as described(16). Values given are in pmoles NP bound/mg DNA/mg protein in 1 h.

[c]Values given are in nmoles NP bound/mg DNA/$5x10^8$ cells in 1 h.

DISCUSSION

Nitropyrenes are demonstrably potent genotoxic agents in both bacterial and mammalian cell assays (2,8,9), and NP itself can generate active species which bind to DNA (6,10,18 and this report). *In vivo* NP is metabolized to compounds which are themselves mutagenic. Nitropyrene phenols were shown to be formed from NP *in vitro* by Aroclor-induced rat liver S9 (19). The virtual absence of NAAP-6/8-OH and of S9-dependent mutagenicity in the urines of germ-free rats indicates that the gut flora plays a considerable role in the formation of this compound, by reduction of NP phenols or hydrolysis of conjugates excreted *via* the bile, thus rendering the aglycones available for reabsorption from the intestine. Urinary excretion of these metabolites implies escape from the entrohepatic circulation cycle, and redistribution from the liver to the kidneys *via* the bloodstream. Thus all vascularly-irrigated organs are

potentially exposed to NP or particularly its more active metabolites; the outcome of such exposure will then be largely dependent on the metabolic susceptibility of the individual organs.

ACKNOWLEDGMENTS

We thank Drs. M. Nishioka and J.C. Chuang, Battelle, Columbus, OH, R.W. Roth, Midwest Research Institute, Kansas City, MO, J.J. Rafter and J.A. Gustafsson, Huddinge University Hospital, Huddinge, B.E. Gustafsson, Karolinska Institute, Stockholm, and M.J. Kohan, HERL, EPA, Research Triangle Park, NC, for their contributions to various aspects of these studies, Ms. Anatra Hunter for typing this manuscript and the National Research Council, Washington, DC, for a Resident Research Associateship Award to L.M.B. Although this information has been reviewed and cleared by the Health Effects Research Laboratory and approved for publication, this article does not necessarily reflect the views of the U.S. Environmental Protection Agency. No official endorsement of any kind should be inferred.

REFERENCES

1. Lofroth, G., Hefner, E., Alfheim, I. and Moller, M. (1980): Mutagenic activity in photocopies. Science 209: 1037-1039.

2. Rosenkranz, H.S., McCoy, E.C., Sanders, D.R., Butler, M., Kiriazides, D.K., and Mermelstein, R. (1980): Nitropyrenes: isolation, identification and reduction of mutagenic impurities in a carbon black and toners. Science, 209: 1039-1043.

3. Nishioka, M.G., Peterson, B.A. and Lewtas, J. (1982): Comparison of nitro-aromatic content and direct-acting mutagenicity of diesel emissions. In: M. Cooke, A.J. Dennis and G.L. Fisher (eds.), Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry, Columbus, OH: Battelle Columbus Press, 603-612.

4. Schuetzle, D., Riley, T.L., Prater, T.J., Harvey, T.M., and Hunt, D.F. (1982), Analysis of nitrated polycyclic aromatic hydrocarbons in diesel particulates, Anal. Chem., 54, 265-271.

5. Wang, C.Y., Lee, M.S., King, C.M. and Warner, P.O. (1980): Evidence for nitroaromatics as direct-acting mutagens of airborne particulates. Chemosphere 9: 83-87.

6. Messier, F., Lu, C., Andrews, P., McCarry, B.E., Quilliam, M.A., and McCalla, D.R. (1981): Metabolism of 1-nitropyrene and formation of DNA adducts in Salmonella typhimurium. Carcinogenesis, 2: 1007-1011.

7. Rosenkranz, E.J., McCoy, E.C., Mermelstein, R., and Rosenkranz, H.S. (1982), Evidence for the existence of distinct nitroreductases in Salmonella typhimurium: Roles in mutagenesis, Carcinogenesis, 3, 121-123.

8. Campbell, J., Crumplin, G.C., Garner, J.V., Garner, R.C., Martin, C.N., and Rutter, A. (1981): Nitrated polycyclic aromatic hydrocarbons: potent bacterial mutagens and stimulators of DNA repair synthesis in cultured human cells. Carcinogenesis, 2: 599-656.

9. Nachtman, J.P. and Woff, S. (1982): Activity of nitropolynuclear aromatic hydrocarbons in the sister chromatid exchange assay with and without metabolic activation. Environ. Mutag. 4: 1-7.

10. King, L.C., Jackson, M., Ball, L.M. and Lewtas, J. (1983): Binding of 1-nitro[^{14}]Cpyrene to DNA and protein in cultured lung macrophages and respiratory tissues. Cancer Letters, 19: 241-246.

11. Ball, L.M., Kohan, M.J., Inmon, J., Claxton, L.D. and Lewtas, J. (1983): Metabolism of 1-nitro[^{14}C]pyrene in vivo in the rat and mutagenicity of urinary metabolites. Cancer Research, Submitted for review.

12. Gustafsson, B.E. (1959): Lightweight stainless steel systems for for rearing germ-free animals. Ann N.Y. Acad Sci 78: 17-28.

13. Ames, B.W., McCann, J.M., and Yamasaki, E. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 31:347-364.

14. Claxton, L.D., Kohan, M., Austin, A.C., and Evans, C. (1982): The Genetic Bioassay Branch Protocol for Bacterial Mutagenesis Including Safety and Quality Assurance Procedures, EPA-600/2-82-008, U.S. Environmental Protection Agency,

Research Triangle Park, NC. pp. 29-31.

15. Inmon, J., Stead, A., Waters, M.D. and Lewtas, J. (1981): Development of a toxicity test system using primary rat liver cells. In Vitro 17: 1004-1010.

16. Jackson, M.A., King, L.C., and Ball, L.M. (1983): A rapid technique for estimating DNA binding, used to evaluate 1-nitropyrene adduct formation. Drug Chem. Toxicol. 6, in press.

17. Ball, L.M., Rafter, J.J., Gustafsson, J.A., B.E., and Lewtas, J. (1983): Metabolism of 1-nitropyrene in germ-free and conventional rats: Role of the gut flora in generation of mutagenic metabolites. In: Extrahepatic Drug Metabolism and Chemical Carcinogenesis, Stockholm, May 1983, edited by J. Rydström et al., Amsterdam, Elsevier Press, pp 295-296.

18. Howard, P.C. and Beland, F.A. (1982): Xanthine oxidase-catalysed binding of 1-nitropyrene to DNA. Biochem Biophys Res Commun, 104: 727-732.

19. El-Bayoumy, K. and Hecht, S.S. (1983): Identification and mutagenicity of metabolites of 1-nitropyrene formed by rat liver. Cancer Res., 43, 3132-3137.